



Time-dependent in-vivo effects of interleukin-2 on neurotransmitters in various cortices: Relationships with depressive-related and anxiety-like behaviour

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ARTICLE INFO

Article history:

Received 3 November 2010

Received in revised form 27 April 2011

Accepted 24 May 2011

Keywords:

Cytokines
Anxiety
Depression
Forced swim test
In-vivo microdialysis
HPLC-EC

ABSTRACT

We investigated the impact of systemically injected IL-2 (2.5 µg/kg, i.p.) on serotonergic and dopaminergic neurotransmission in various cortical areas by in-vivo microdialysis. IL-2 lastingly reduced extracellular 5-HT levels in the medial prefrontal (−75%), occipital (−70%), and temporal cortices (−45%), whereas dopamine was only moderately reduced in the medial prefrontal cortex.

Based on the serotonergic time profile, we conducted further experiments to test for acute and delayed (2 h post injection) depressive-related effects of systemic IL-2 (0–5.0 µg/kg) in a forced swim test and delayed effects on anxiety-like behaviour in the elevated plus-maze. IL-2 had dose-dependent effects on depressive-related behaviour after delayed but not acute testing, but no effects on anxiety-like behaviour.

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1. Introduction

Cytokines are suggested to modulate emotional and motivational processes (Kiecolt-Glaser et al., 2002; Anisman et al., 2005) and to be involved in certain psychopathologies, namely depression and anxiety disorders (Anisman and Merali, 1999; Dantzer et al., 2008). Blood cytokine levels differ between healthy controls and patients diagnosed with affective and motivational disorders, such as major depression (Seidel et al., 1995; Myint et al., 2005; Tsao et al., 2006), bipolar disorder (Kim et al., 2007a), dysthymia (Anisman and Merali, 1999), and panic disorder (Rapaport and Stein, 1994). Furthermore, the combination of interleukin-2 (IL-2) and IFN-α immunotherapy in cancer patients (Capuron et al., 2000; Capuron et al., 2001) can lead to symptoms of depression. Also, administration of IL-2 during cancer immunotherapy led to a decrease of blood tryptophan (essential

amino acid precursor of 5-HT), and an increase in urine kynurenine, a metabolite of tryptophan (Brown et al., 1989). Follow-up studies supported this potential association between IL-2, decreased tryptophan, and depressive symptoms (Capuron et al., 2002a; Capuron et al., 2003). Imaging studies furthermore showed that hepatitis C patients treated with low doses of IFN-α had hypometabolism in the prefrontal cortex, measured by PET scan, which was correlated with an increase in depression score; this was accompanied by hypermetabolism in the putamen (as a part of the basal ganglia) and left occipital cortex (Juengling et al., 2000). Capuron et al. (2007) partially supported these results: Cancer patients who received IFN-α therapy also had decreased metabolism in the prefrontal cortex and the basal ganglia. Moreover, subjective feelings of energy were negatively correlated with glucose metabolism in the left nucleus accumbens and the putamen.

Several animal studies have demonstrated impacts of cytokines on central neurotransmission relevant for affective motivated behaviours (Anisman et al., 2005). Apart from the pro-inflammatory cytokines IL-1, IL-6, or tumour necrosis factor-α (TNF-α), IL-2 has been shown to be involved in the modulation of various neurotransmitters in the brain (Hanisch, 2001; Dunn et al., 2005b). For example, IL-2 administration influenced DA levels and turnover in the prefrontal cortex (Zalcman et al., 1994), nucleus accumbens (Anisman et al., 1996; Song et al., 1999), neostriatum, and substantia nigra (Lacosta et al., 2000). Other animal studies provided evidence for an increase of IL-2 on 5-HT levels

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in the hippocampus, and a decrease in the prefrontal cortex post mortem (Lacosta et al., 2000). In-vivo, intracerebroventricular IL-2 increased 5-HT and its metabolite 5-hydroxyindoleacetic acid (5-HIAA) in the hippocampus (Pauli et al., 1998), and systemic IL-2 increased 5-HIAA in the nucleus accumbens (Song et al., 1999).

In contrast, studies on IL-2 and behaviour in animal models relevant for depression or anxiety are still scarce. Concomitant to human findings, IL-2 also led to alterations in depressive-related or anxiety-like behaviours. For example, systemic IL-2 was reported to alter exploration of and approach to a novel stimulus (Zalcman et al., 1998; Lacosta et al., 1999; Zalcman, 2001). Furthermore, whilst acute administration of IL-2 did not influence hedonic behaviour measured by the consumption of palatable food (Anisman and Merali, 1999), repeated IL-2 infusion markedly reduced consumption (Anisman et al., 2002). Systemic IL-2 also impaired responding for rewarding brain stimulation in the medial forebrain bundle, which can be taken as a measure of anhedonia (Anisman et al., 1996). However, IL-2 has not been tested in other paradigms measuring depressive-related behaviour, like the forced swim test (FST).

Previously, we found IL-2 mRNA levels in the striatum and prefrontal cortex to be correlated with individual anxiety-like behaviour (Pawlak et al., 2003; Pawlak et al., 2005). Experiments showed anxiogenic-like trends for elevated plus-maze (EPM; Pawlak and Schwarting, 2006), and open field behaviour effects (Karrenbauer et al., 2009) when IL-2 was injected into the striatum (ventral and dorsal part). However, there are no studies which analysed the impact of peripherally administered species-specific IL-2 on neurotransmission in the living animal and corresponding behaviours. Thus, in the first part of this study, we focused on systemic IL-2 and its effects on neurotransmission in several cortical areas (medial prefrontal, occipital, and temporal cortex), as measured by in-vivo microdialysis (Exp. 1). The second part consists of two behavioural experiments: Experiment 2 investigated the impact of IL-2 on depressive-related behaviour measured by the forced swim test (FST) upon acute (Exp. 2a), and delayed IL-2 treatment (Exp. 2b). Finally, delayed IL-2 effects on anxiety-like behaviour were tested by the elevated plus-maze (EPM; Exp. 3).

2. Methods

2.1. Material and methods

All experiments were conducted in conformity with the Animal Protection Law of the Federal Republic of Germany.

2.1.1. Animals and surgery

In each of the following experiments separate groups of animals were used.

2.1.1.1. Microdialysis and high-performance liquid chromatography. Male outbred Wistar rats (animal facility, University of Düsseldorf, Germany), weighing 349 g (+ 13.5, SD) before surgery, were used. Until surgery they were housed four animals per cage under standard laboratory conditions, with a reversed light–dark rhythm (light on from 19:00 to 7:00) with food and tap water provided ad libitum. For surgery, rats were deeply anaesthetised with a mixture of 0.9 ml/kg Ketavet (containing 100 mg/ml Ketamine; Pharmacia and Upjohn, Germany) and 0.4 ml/kg Rompun (containing 20 mg/ml Xylazine; Bayer, Germany) and placed in a Kopf stereotaxic frame. Three guide cannulae with a thread on the top were aimed at the medial prefrontal cortex, mPFC (AP: +2.8, ML: +/–1.0, DV: –2.4, angle: 5° from midline), the occipital cortex, OccC (A: –6.8; L: +4.5; V: –1.8; angle: +20°), and the temporal cortex, TempC (A: –3.8; L: +4.5; V: –3.5; angle: +20°; all coordinates relative to bregma; Paxinos and Watson, 1986) and fixed to the skull with two stainless steel screws and dental cement (Müller et al., 2007). To prevent post-operative pain, 100 µl Novaminsulfon-

ratiopharm (containing 500 mg/ml Metamizol-sodium) was administered p.o. after rats awoke from anaesthesia. After surgery, the animals were housed individually. They were handled daily and were allowed to recover for at least 4 days.

2.1.1.2. Forced swim test. Seventy-nine (Exp. 2a: n=39; Exp. 2b: n=40) male outbred Wistar Unilever rats (Harlan Winkelmann, Borcheln, Germany) were used, weighing 269 ± 7.4 g in Experiment 2a and 261 ± 10.9 g in Experiment 2b on arrival in the lab. They were housed in groups of four in acrylic cages (38 × 20 × 59 cm). The animals were kept under standard laboratory conditions under a 12 hour light–dark cycle (lights on: 7:00–19:00) and with food and tap water available ad libitum.

2.1.1.3. Elevated plus-maze. Seventy-six male outbred Wistar Unilever rats (Harlan Winkelmann) were used, weighing 282 ± 7.7 g on arrival in the lab. The animals were kept under the same conditions as in Experiment 2a and 2b.

2.1.2. In-vivo microdialysis (Exp. 1)

2.1.2.1. General procedure. On the day of the experiment the animals were anaesthetised with 1.25 g/kg urethane (i.p.) and microdialysis probes of concentric design (membrane length: mPFC: 2 mm; OccC and TempC: 3 mm; 6 kDa molecular cut off) were inserted into the guide cannulae and fixed to the thread (probe construction described in Boix et al., 1995). Ex-vivo recovery for these probes at the used perfusion conditions were 13.7% for DA and 21.6% for 5-HT. After probe insertion the animal was placed on a heating pad within a sound and light isolated chamber kept under red light conditions (2.6 lx; Müller et al., 2007). Body temperature was maintained during the experiment between 36.5 and 37.5 °C by a temperature controller. Animals received 0.2 ml phosphate buffered saline (PBS) every 20 min via a catheter in the intraperitoneal cavity to maintain body fluid balance, or additional urethane, when required. The microdialysis probes were connected to a microinfusion pump (CMA 100, Carnegie, Sweden) on top of the chamber and were perfused with artificial cerebrospinal fluid (aCSF) containing Na⁺ 147 mmol, K⁺ 4 mmol, Ca²⁺ 2.2 mmol, Cl[–] 156 mmol, pH = 7.4 at room temperature. The perfusion flow was set at 1.08 µl/min and was allowed to stabilise for at least 2 h. Then, samples were collected every 20 min into vials containing 2 µl of 0.1 M perchloric acid and 500 pg dihydroxybenzylamine (DHBA) as internal standard (Pum et al., 2008). After obtaining stable levels for 5-HT and DA, three 20 min samples were taken as baseline. Subsequently, rats received an i.p. injection with either IL-2 (2.5 µg/kg) or PBS. The injection volume was 1 ml/kg. Thereafter, sampling continued for 3 h (9 samples).

2.1.2.2. Analytical procedure. The samples were immediately assayed after collection using high-performance liquid chromatography with electrochemical detection. The column was an ET 125/2, Nucleosil 120–5, C-18 reversed phase column (Macherey-Nagel, Germany) perfused with a mobile phase composed of 75 mM NaH₂PO₄, 4 mM KCl, 20 µM EDTA, 1.5 mM sodium dodecylsulfate, 100 µl/l diethylamine, 12% methanol and 12% acetonitril adjusted to pH 6.0 using phosphoric acid (modified from: Chen and Reith, 1994). The electrochemical detector (Intro, Antec, Netherlands) was set at 500 mV vs. an ISAAC reference electrode (Antec, Leyden, Netherlands) at 30 °C. This set-up allows the measurement of 5-HT and DA in cortical samples. The detection limit of the assay was 0.1 pg for 5-HT and DA with a signal-to-noise ratio of 2:1. Neurochemical data were not corrected for recovery.

2.1.2.3. Histological analysis. The animals were deeply anaesthetised with 0.5 ml Nembutal (containing 60 mg/ml pentobarbital; Sanofi, France) and transcardially perfused with PBS followed by 10%

phosphate buffered formalin. The brains were removed, sliced on a cryotome, and stained with cresyl-violet for microscopic analysis of probe placement. Only animals with placement within the respective brain areas were considered for data analysis.

2.1.3. Behavioural tests (Exp. 2a/b, Exp. 3)

Upon arrival, animals were weighed daily until the end of the experiment. Also, they were gentled in a standardised procedure during which each rat was touched and picked up for 5 min on three consecutive days (day 2–4). In Experiment 2a and 2b, animals were routinely exposed on two consecutive days both, to an open field (OF, day 5 and 6, 10 min each) and to an elevated plus maze (EPM, day 9 and 10, 5 min each; data not shown). In each group, rats were screened for their behavioural response to a novel open field and assigned to the groups based on comparable horizontal locomotor activity (distance travelled; data not shown). All tests were conducted during the light cycle between 09:00–18:00 h.

2.1.3.1. Forced swim test (FST, Exp. 2a/b). Three days after the screening tests, the animals were tested in the FST on two consecutive days. On day 1 (FST 1, 15 min), animals were tested without pharmacological treatment, whilst 24 h later and prior to the second test (FST 2, 5 min) IL-2 was injected. In Experiment 2a the animals were tested acutely (5 min), whereas in Experiment 2b they were tested 2 h after injection. Different groups of rats (9–10 rats/group) received either 1, 2.5, or 5 µg/kg of IL-2, or sterile PBS. Pharmacological treatment and behavioural testing were conducted in separate rooms. Carrier-free recombinant rat IL-2 (R&D Systems, USA) was used, which was delivered as a 50 µg lyophilised filtered solution in 20 mM of ammonium acetate. Appropriate volumes of sterile PBS (Dulbecco's, Gibco®, Invitrogen Germany) were added to obtain aliquots containing 1, 2.5, or 5 µg of IL-2. All groups received a single systemic (i.p.) injection of either IL-2 (1, 2.5, or 5 µg/kg), or PBS.

The FST procedure was derived from Porsolt et al. (1977, 1978), but with modifications regarding water depth, temperature, and procedure (Cryan et al., 2002). In detail, a square glass tank (25 × 25 × 60 cm) was filled with 27 °C water to a depth of 40 cm. This depth was chosen so that the animals could swim and float without hind limbs or tail touching the bottom of the tank. The apparatus was cleaned after each trial with 0.1% acetic acid solution and refilled with fresh water.

For FST testing, each rat was placed gently into the tank. After testing the following behavioural measures were scored from videotape by two observers (inter-rater reliability $r = .90$ $P \leq .001$) blind with respect to treatment: 1) immobility, defined as the time the rat made only slow and minimal vertical movements (slow sinking, calm movements just necessary to get back to surface during which the paws did not break the water surface), and 2) struggling, defined as the time the rat showed intense movements with all four paws and with the forepaws touching the wall of the tank, or breaking the water surface. These movements are mainly vertical, and the animals typically make excessive movements to keep the nose above the water surface. In line with the literature (Porsolt et al., 1977), immobility during FST 2 (5 min) was taken as the index of depressive-related behaviour. In addition, behaviour during the same time period of FST 1 was taken as baseline values.

2.1.3.2. Elevated plus-maze (EPM, Exp. 3). The animals were tested on the EPM on two consecutive days. Pharmacological treatment took place only prior to EPM 1, whilst 24 h later (EPM 2) animals were tested in the drug-free state. All groups received a single systemic (i.p.) injection of either IL-2 (1, 2.5, or 5 µg/kg; $n = 19$ each), or PBS ($n = 18$).

Testing was carried out in two identical set-ups, which were situated in two separated chambers. They were made of grey plastic and consisted of two opposed open arms (50 × 10 cm), two opposed closed arms (50 × 10 × 40 cm), and a middle section (10 × 10 cm) in

the centre. A small raised edge (5 mm) surrounding the open arms prevented animals from falling off. The apparatus was elevated 50 cm above the floor and behaviour was monitored by a video camera from above. The EPM was illuminated by four white bulbs, producing a light intensity of 30 lx in the centre.

For testing, every animal was carried from the animal room to the test room in an extra cage. This transportation cage was cleaned thoroughly by 0.1% acetic acid solution before each animal. Testing always lasted 5 min. Thereafter, the rat was brought back to the animal room.

The following behavioural measurements were scored from videotape by trained observers, who were blind with respect to the treatments: 1) time spent on the open arms, 2) time spent on the closed arms (time was counted when all four paws were placed on one arm), 3) number of rearings, separately for closed and open arms and the middle, 4) number of open and closed arm entries (entry was counted when all four paws were placed in one arm), 5) latencies until first open and closed arm entry, respectively, and 6) risk assessment time. Risk assessment was counted when the animal was situated in a closed arm (at least with one paw), but explored the open arm with its head, i.e. the eyes of the animal had to pass the line between the middle section and the open arm.

2.1.4. Additional homeostatic measures for sickness behaviour

Since a number of cytokines can reliably induce inflammation and sickness behaviour, body temperature and body weight were recorded as systemic physiological measures. Rectal temperature was measured only in Experiment 2 using a digital thermometer (Präzisionsthermometer, Testo, Germany) 5 min after FST testing. Body weight was recorded in each experiment, but was exemplary analysed as a measure for sickness behaviour only in Experiment 3. There, data were collected directly before IL-2 treatment on EPM 1 and 24 h later (EPM 2).

2.1.5. Statistics

The neurochemical data were expressed as percentage of the baseline samples, which were taken as 100%. The data were analysed by two-way repeated measures ANOVAs with treatment and time as factors. In order to compare single time points, Tukey's HSD tests were used. To determine the absolute magnitude of the neurochemical effects over time, areas under the curve (AUC) were calculated by summing the values of the samples after the injection, and were compared vs. control.

Behavioural coding was executed by trained observers blind to treatment. Identically to other studies behavioural data were analysed with univariate ANOVAs for each test day. Post hoc tests were performed using least significant difference (LSD) tests. ANOVA results revealing a statistical trend ($P < .10$) were also analysed by post hoc comparisons (Pawlak and Schwarting, 2006). The rationale is that the overall F is not required in order to conduct multiple comparisons (Wilcox, 1987; Howell, 1992).

Data are expressed as mean + SEM. All P -values are two-tailed and taken as statistically significant when $P \leq .05$.

3. Results

3.1. Experiment 1: in-vivo microdialysis

3.1.1. Histological results

After exclusion of incorrect probe placements, the following group sizes were obtained: mPFC saline $n = 7$, IL-2 $n = 6$; OccC: saline $n = 5$, IL-2 $n = 5$; TempC: saline $n = 7$, IL-2 $n = 5$. For probe placements see Fig. 1.

3.1.2. Basal neurotransmitter levels

Basal levels of 5-HT were 1.62 ± 0.18 pg (mean \pm S.E.M. in 20 µl) in the mPFC ($n = 13$), 4.19 ± 0.78 pg in the OccC ($n = 10$), and $2.42 \pm$

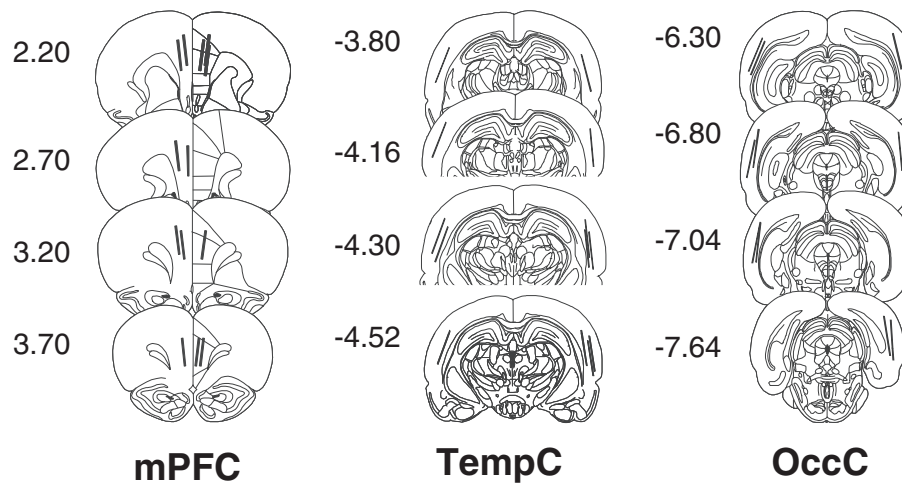


Fig. 1. Localisation of microdialysis probes within the medial prefrontal cortex (mPFC), temporal cortex (TempC), and occipital cortex (OccC), based on the stereotaxic atlas by Paxinos and Watson (1986). Caption refers to the anterior–posterior coordinates relative to bregma.

0.32 pg in the TempC ($n=12$). Basal DA levels were 1.27 ± 0.16 pg, 0.65 ± 0.15 pg, and 1.02 ± 0.22 pg for mPFC ($n=12$), OccC ($n=10$), and TempC ($n=12$), respectively. These basal extracellular levels did not differ between groups (P -values >0.05).

3.1.3. IL-2 effects on cortical 5-HT levels

IL-2 significantly decreased 5-HT levels in the mPFC (Fig. 2a). Statistical analysis showed a significant effect of treatment ($F=63.23$, $P<0.001$), time ($F=5.26$, $P<0.001$), and a significant treatment \times time interaction ($F=8.97$, $P<0.001$). Single time point comparisons vs. saline revealed a significant decrease in 5-HT levels in the 80 min ($P=0.009$), 100 min ($P=0.008$), and in the 120–180 min ($P<0.001$) samples after injection. These findings were confirmed when AUC values were considered ($t=-7.93$; $P<0.001$).

There was also a significant decrease of 5-HT levels in the OccC (Fig. 2b). Statistical analysis showed a significant effect of treatment ($F=26.09$, $P<0.001$), time ($F=5.43$, $P<0.001$), and a significant treatment \times time interaction ($F=7.53$, $P<0.001$). Single time point comparisons vs. saline revealed a significant decrease in 5-HT levels in the 40 min ($P=0.034$), 60 min ($P=0.003$), 80 min ($P=0.019$), 100 min ($P=0.002$), and in the 120–180 min ($P<0.001$) samples after injection. These findings were confirmed when AUC values were considered ($t=-5.11$; $P<0.001$).

Finally, IL-2 significantly decreased 5-HT levels in the TempC (Fig. 2c). Statistical analysis showed a significant effect of treatment ($F=21.08$, $P=0.001$), time ($F=2.34$, $P=0.013$), and a significant treatment \times time interaction ($F=3.84$, $P<0.001$). Single time point comparisons vs. saline revealed a significant decrease in 5-HT levels in the 80 min ($P=0.007$), 120 min ($P=0.035$), 140 min ($P=0.005$), 160 min ($P=0.040$), and 180 min ($P=0.002$) intervals after injection. These findings were confirmed when AUC values were considered ($t=-4.62$; $P=0.010$).

3.1.4. IL-2 effects on cortical dopamine levels

IL-2 significantly decreased DA levels in the mPFC (Fig. 3a), but this effect was less pronounced than the IL-2 effect on 5-HT. Statistical analysis showed a significant effect of treatment ($F=6.82$, $P=0.026$), but no time effect, or treatment \times time interaction ($P>0.05$). Single time point comparisons did not yield significant differences vs. saline ($P>0.05$). However, an overall treatment effect was confirmed when AUC values were considered, showing that IL-2 treatment signifi-

cantly reduced DA levels in the mPFC compared to saline controls ($t=-2.58$; $P=0.028$). In contrast, IL-2 did not affect DA levels in the OccC and TempC (Fig. 3b and c), where neither analysis of variance nor AUC value comparisons revealed significant differences vs. saline ($P>0.05$).

3.2. Experiment 2: behaviour in the forced swim test

In Experiment 1, we had obtained a clear reduction of 5-HT in different cortical areas, which followed a specific time profile. There is evidence that decreased 5-HT is associated with symptoms of depression (Coppin, 1967). Also, a negative correlation between 5-HT levels and depressive symptoms in IFN- α -induced depression was shown (Bonaccorso et al., 2002). Furthermore, depression and also cytokine-induced depressions can successfully be treated with selective serotonin reuptake inhibitors (SSRIs; Hauser et al., 2002; Musselman et al., 2001; Capuron et al., 2002b,c). On the basis of the postulated link between 5-HT, depression, and cytokines, we analysed the impact of acute, or 2 h delayed, systemic IL-2 on depressive-related behaviour measured by the FST. Here, the first test (FST 1) served as a baseline, and the second test (FST 2) as the drug test.

3.2.1. Experiment 2a: no effects of IL-2 on acute testing in the forced swim test

In the baseline test (FST 1), i.e. without drug treatment, we obtained no group differences in immobility ($F=0.39$, $P=.760$; Fig. 4a), or struggling ($F=1.20$, $P=.325$; Fig. 4b). Also, and in contrast to Experiment 2b (see below), we observed no differences between IL-2 or PBS treatments in the FST 2 performed acutely after injection (immobility: $F=0.80$, $P=.502$; struggling: $F=.68$, $P=.573$).

3.2.1.1. Body temperature. There were no significant differences between the IL-2 treated groups and the control group ($\leq F=1.08$, $\geq P=.371$).

3.2.2. Experiment 2b: effects of IL-2 on delayed testing in the forced swim test

There were no baseline (FST 1) differences between groups, neither in immobility ($F=0.22$, $P=.881$), nor struggling ($F=0.24$, $P=.870$; data not shown). During FST 2, i.e. 2 h after injection, we found a significant group effect in immobility ($F=3.75$, $P=.020$; Fig. 4c). LSD post hoc analysis revealed significantly more immobility

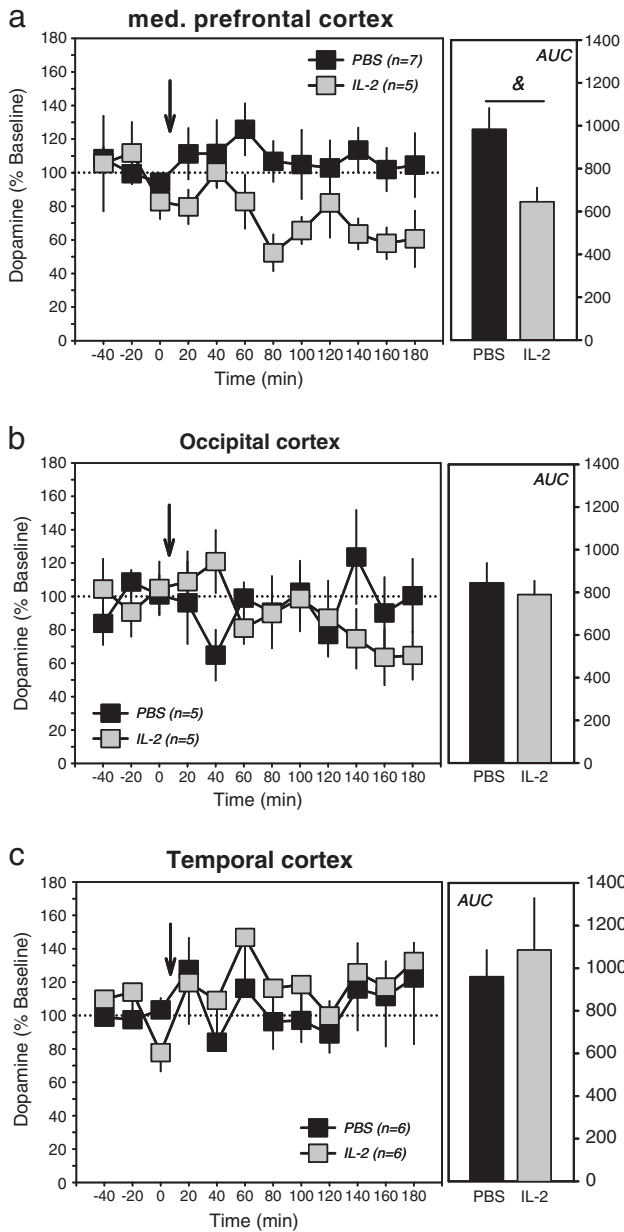


Fig. 2. Extracellular 5-HT (serotonin) levels in the medial prefrontal cortex (a), occipital cortex (b), and temporal cortex (c) after interleukin-2 (2.5 µg/kg, i.p.) injection (* $P < 0.05$, + $P < 0.01$, # $P < 0.001$, two-way ANOVA followed by Tukey's HSD test vs. saline). Boxes: AUC values ([§] $P < 0.01$, [§] $P < 0.001$, two-tailed t -test).

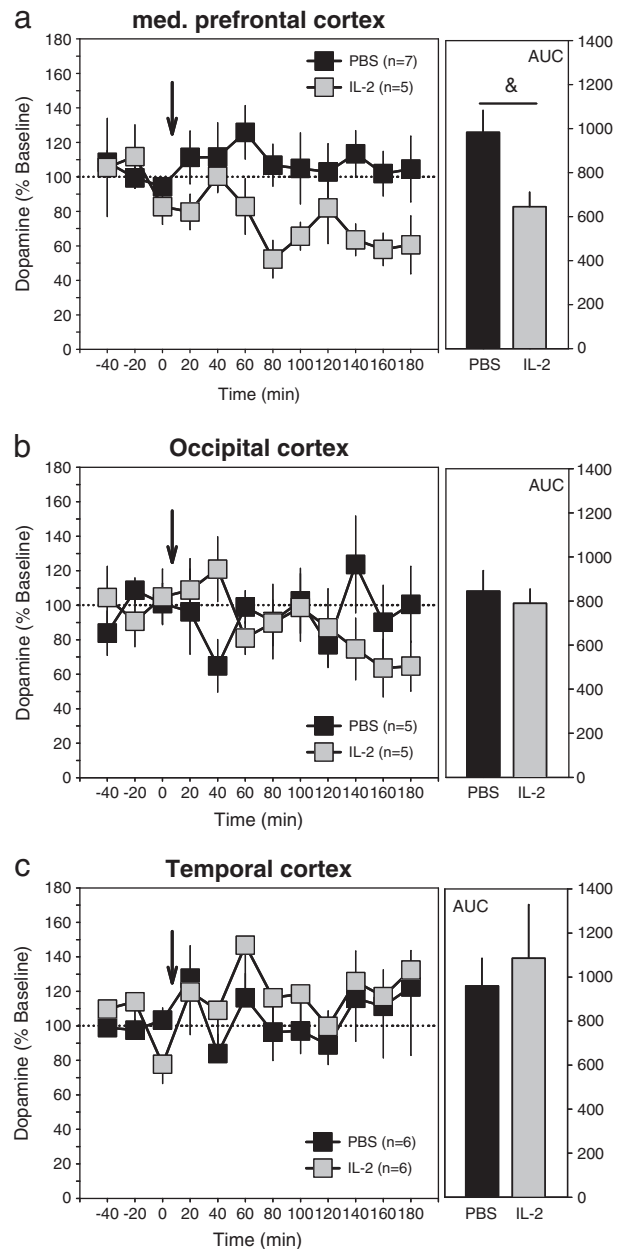


Fig. 3. Extracellular DA (dopamine) levels in the medial prefrontal cortex (a), occipital cortex (b), and temporal cortex (c) after interleukin-2 (2.5 µg/kg, i.p.) injection. Boxes: AUC values ([§] $P < 0.05$, two-tailed t -test).

in animals treated with the lowest IL-2 dose (1 µg/kg, $P = .049$) compared to the control group, and the group treated with the highest IL-2 dose (5 µg/kg; $P = .003$). Furthermore, animals treated with the intermediate dose of IL-2 (2.5 µg/kg) showed increased immobility compared to the highest dose ($P = .045$).

An opposite behavioural pattern was found in struggling behaviour, but only with a trend for a group effect ($F = 2.61$, $P = .067$; Fig. 4d). Post hoc analysis also showed a trend for decreased struggling behaviour in the 1 µg IL-2 group compared to controls ($P = .100$). In contrast, the group treated with the highest dose of IL-2 (5 µg/kg) showed significantly more struggling than the lowest IL-2 dose (1 µg/kg; $P = .011$), and a trend compared to the intermediate IL-2 dose (2.5 µg/kg; $P = .091$).

3.3. Experiment 3: no effects of IL-2 on delayed elevated plus-maze behaviour

Here, we wanted to test whether systemic IL-2 also had effects on anxiety-like avoidance behaviour in the EPM. Since the previous FST experiments showed that IL-2 had delayed, but not acute, behavioural effects, we restricted our analysis to the delayed condition, i.e. EPM testing at 2 h after injections. Also, we repeated the test on the consecutive day to examine whether the treatments might have even longer lasting effects (Karrenbauer et al., 2009), or behavioural trends (Pawlak and Schwarting, 2006) as was previously found in case of intrastriatal injections.

Systemic IL-2, however, had no effects on delayed testing for anxiety-like behaviour, since there were no differences between IL-2 and PBS treated animals regarding the time spent on the open arms,

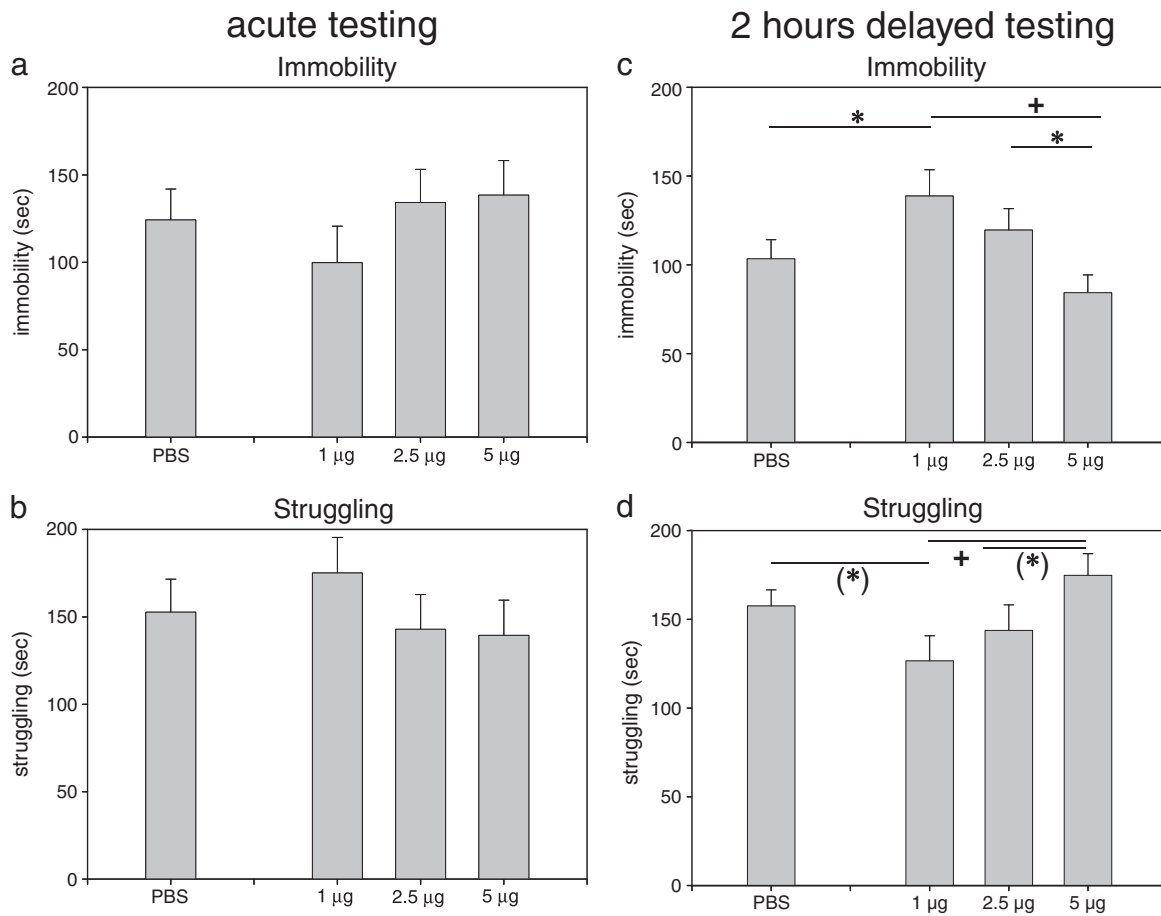


Fig. 4. Effects of peripheral IL-2 on immobility and struggling behaviour in a forced swim test. This test was performed acutely (a and b) and 2 h (c and d) after injections of IL-2 (1, 2.5, or 5 µg/kg) or PBS. Results are presented as means±SEM (* $P < 0.05$; + $P < 0.01$; (*) $P < 0.1$). Please refer to the text for the statistical analyses.

neither during the first (EPM 1: $F = .55$; $P = .649$) nor the second test (EPM 2: $F = 1.07$; $P = .369$). When data were expressed as percent time spent on the open arms, there were also no significant differences between the IL-2 treated and the control group (EPM 1: $F = .03$; $P = .994$; EPM 2: $F = 1.11$; $P = .350$; Fig. 5a). Furthermore IL-2 had neither effects on time spent in the closed arms or on the percent time spent on the closed arms ($\leq F = 1.11$; $\geq P = .350$; see Fig. 5b) nor on the measurement for locomotion, namely entries into arms (EPM 1: $F = 1.77$; $P = .161$; EPM 2: $F = .39$; $P = .764$; Fig. 5c).

In all other variables (EPM 1, EPM 2) there were no significant differences between IL-2 and control groups ($\leq F = 2.11$; $\geq P = .106$).

3.3.1. Body weight

There were no significant differences between IL-2 treated and control animals on body weight before ($F = 0.20$; $P = .899$) and 1 day after IL-2 treatment ($F = 0.24$; $P = .869$).

4. Discussion

In the first experiment, we focused on systemic IL-2 and its effects on neurotransmission in several cortical areas as measured by in-vivo microdialysis. Our results showed that 2.5 µg/kg of IL-2 excessively reduced cortical 5-HT levels, whereas DA was comparably mildly affected. Importantly, the 5-HT effects developed over time, that is, they became significant after around 80 min, most pronounced after about 120 min, and sustained at least until the end of data collection 180 min upon IL-2 treatment. To test for functional consequences, two

behavioural experiments were then performed, where we tested A) IL-2 effects on depressive-related behaviour using the FST (Exp. 2a/b), and B) effects on anxiety using the EPM (Exp. 3). Based on the temporal neurochemical profiles, we expected that IL-2 effects should be more pronounced around 2 h after injection (delayed) as compared to acute testing, i.e. shortly after IL-2 administration. The main result unexpectedly showed that only the low dose of IL-2 (1 µg/kg) led to depressive-related effects, but that the dose of IL-2 that was shown to reduce serotonin release (2.5 µg/kg) failed to alter depressive-related behaviour. Further according to our hypothesis, these effects were obtained only after delayed but not after acute testing. Against our expectations, we found no effects of systemic IL-2 on anxiety-like behaviour.

4.1. Neurochemistry

Older work had indicated that IL-2 can lead to reduced DA release in the nucleus accumbens (Anisman et al., 1996), but there has been only one study so far, which showed that systemically injected IL-2 affects neurotransmission in the living rat as measured by in-vivo microdialysis in the nucleus accumbens (Song et al., 1999). There, repeated injection of human IL-2 (i.p.) led to a reduction in DAergic activity (DA and homovanillic acid) and serotonergic turnover as measured by the 5-HT metabolite 5-HIAA. Another study (Pauli et al., 1998) showed that rather high i.c.v. doses (500 ng) of IL-2 led to a marked increase in hippocampal levels of 5-HT and 5-HIAA. Our work adds new evidence in showing that a single systemic injection of a comparably low dose of IL-2 (2.5 µg/kg) can be sufficient to decrease

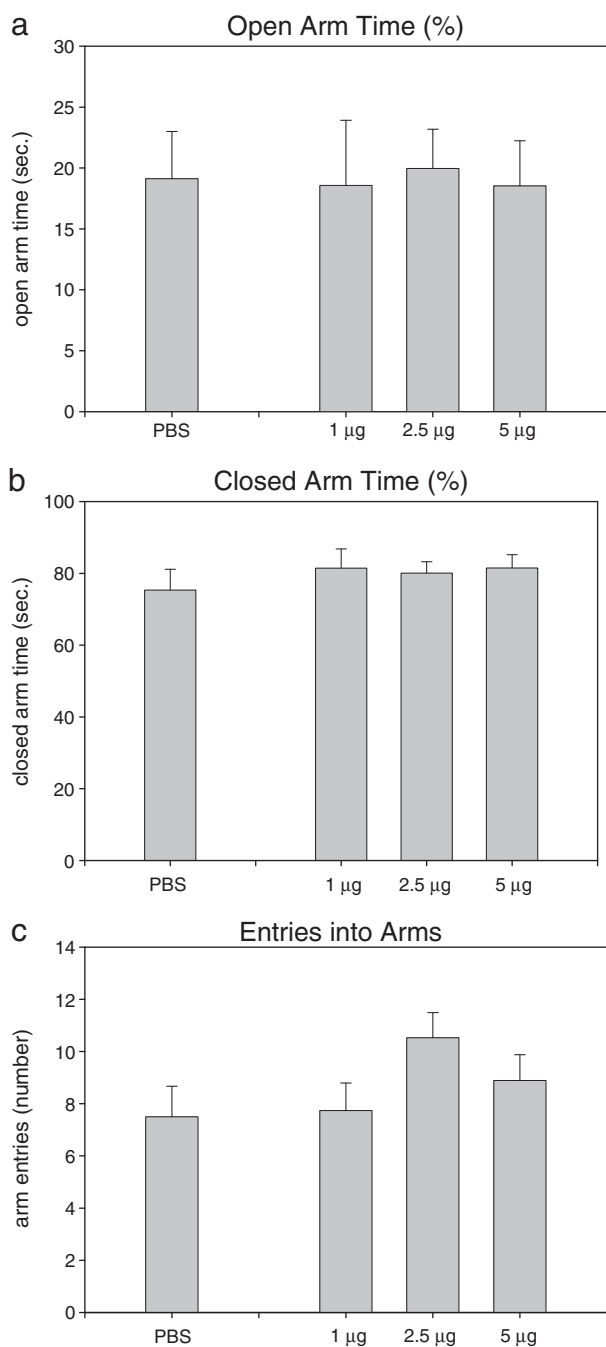


Fig. 5. Effects of peripheral IL-2 on behaviour in an elevated plus-maze (EPM). Behaviour during EPM1 was tested 2 h after injections of IL-2 (1, 2.5, 5 µg/kg), or PBS. Results are presented as means \pm SEM (* P <0.05; + P <0.01). Please refer to the text for the statistical analyses.

extracellular 5-HT in various cortices (mPFC, occipital, and temporal cortex), and can partially decrease also DA (mPFC).

There are at least two possible mechanisms by which IL-2 might have affected 5-HT: First, via reducing the availability of tryptophan. There is evidence for a link between cytokines and tryptophan levels (Capuron et al., 2002a; Miura et al., 2008) in that IFN- α and IL-2 can increase IDO (indoleamine 2,3-dioxygenase), the first enzyme in the kynurenine pathway, which degrades and converts tryptophan to kynurenine and then to quinolinic acid (Carlin et al., 1987). Thus, an overexpression of IDO may have resulted in decreased local availability of tryptophan, and consequently, to reduced synthesis of 5-HT. Second, pro-inflammatory cytokines like IL-1 β (Ramamoorthy

et al., 1995), TNF- α (Mössner et al., 1998), and IFN- α (Morikawa et al., 1998) can increase levels of the 5-HT transporter (5-HTT), which then may lead to increased re-uptake of 5-HT into the cell, and thus to a decrease of extracellular 5-HT. However, there are no data on the influence of IL-2 on this transporter. Taken together, the mechanism for our rapid and massive cortical 5-HT suppression remains unknown.

4.2. Depressive-related behaviour

Progressive numbers of studies showed relationships between inflammatory processes, cytokines, and depression (Bonaccorso et al., 2002; Wichers and Maes, 2002). For example, Capuron et al. (2000) have shown that IFN- α and IL-2 immunotherapy in combination with chemotherapy led to depressive symptoms compared to control patients who received only chemotherapy. Follow-up studies showed that these cytokine-induced depressive symptoms could be attenuated by antidepressant drugs (SSRIs; Leonard, 2001; Musselman et al., 2001; Capuron et al., 2002b; Hauser et al., 2002) and that these symptoms were associated with decreased tryptophan levels (Capuron et al., 2002a; Capuron et al., 2003). However, there are also studies (e.g. Steptoe et al., 2003; Lesperance et al., 2004; Marques-Deak et al., 2007) that found no correlation between cytokines, depression, and antidepressant treatment.

To further address this controversial evidence, we analysed the impact of peripherally injected IL-2 on depressive-related behaviour as measured by the FST (Exp. 2). We found dose-dependent effects of IL-2 on immobility in the FST, when tested 2 h post injection, with an increase in depressive-related behaviour in case of the lowest dose (1 µg/kg) and a decrease in case of the highest (5 µg/kg). So far, there is only scarce evidence for depressive-related behaviour induced by cytokines in the FST. Dunn and Swiergiel (2005a) tested mice treated with different doses of IL-1 β in the FST. They found that IL-1 β (1 µg) increased immobility behaviour after a post-injection delay of around 110 min. Albeit the fact that they used a different species and a different cytokine compared to our study, they also found effects after a post-injection delay.

To our knowledge there is no previous study in which the impact of IL-2 on depressive-related behaviour was analysed in the FST, and only scarce evidence is available on other depressive-related paradigms in animals (Anisman et al., 1996, 1998; Dunn et al., 2005b). For example, Anisman et al. (1998) showed that systemic IL-2 (1 µg), disrupted rewarding intracranial self-stimulation in the lateral hypothalamus but did not provoke alterations in reward unrelated behaviour.

We interpret our data as follows: the fact that peripherally injected IL-2 led to dose-dependent depressive-related effects in the FST only when tested 2 h after the injection, may be due to the time-dependent reduction of central 5-HT, and perhaps also DA. Possibly, the same holds for the phenomenon of cytokine-induced depression during cytokine therapy in cancer patients. Interestingly, our effects were seen in cortical structures (prefrontal, occipital), which according to human imaging techniques are involved in depression (Cummins, 1993; George et al., 1993; Drevets et al., 1997), in general, and cytokine-induced depression (Juengling et al., 2000; Capuron et al., 2007), in specific. Still, one might wonder why IL-2 caused an increase in immobility in case of the lowest but not the highest dose. Actually, however, this is not an unexpected finding, since multiphasic response patterns are in accord with the cytokine literature (Montkowski et al., 1997), and with our previous experiments (Pawlak and Schwarting, 2006).

More unexpected is the finding, that the dose of 2.5 µg/kg of IL-2 was highly effective to reduce extracellular levels of cortical 5-HT, but did not lead to behavioural effects in the FST. This discrepancy is probably due to methodological differences between the neurochemical and the behavioural experiments, which were conducted

differently. First, we tested anaesthetized rats, which may differ pharmacokinetically from awake animals, and future microdialysis experiments will be required here using awake, freely-moving rats. On the other hand, we tested the animal hours after the induction of the anaesthesia, when anaesthetic effects on drug-induced neurotransmission seem to be minimal (De Souza Silva et al., 2007). Secondly, the microdialysis study was conducted in the dark phase, whereas the behavioural tests were performed in the light phase of the artificial light/dark cycle. Similar to behaviour, the release of cytokines and neurotransmitters is dependent on circadian rhythm (Lissoni et al., 1998; Vitkovic et al., 2000; Carobrez and Bertoglio, 2005). However, it is unknown how cytokine-induced neurotransmitter releases may be influenced by circadian rhythm and how this might be expressed behaviourally. Finally, we tested only one dose of IL-2 neurochemically. In further studies it is essential to add more doses of IL-2 to obtain a dose–response curve. Despite these limitations, our findings hint at pronounced time-, site-, and transmitter-dependent neurochemical consequences of systemically administered IL-2.

Finally, one has to point out that the FST models only few aspects of the clinical disease, and some, e.g. worries and dysfunctional thoughts, cannot be addressed in such basic rodent behavioural paradigms. Therefore, future studies should also include other depressive-related behavioural aspects and tests, for example, the ambiguous-cue test (Enkel et al., 2010), learned helplessness (Cryan et al., 2002), break point paradigms (Hodos and Kalman, 1963; Schneider et al., 2010a), or anhedonia as tested in the sucrose consumption test (Papp et al., 1991).

4.3. Anxiety-like behaviour

Clinically, a high co-morbidity between anxiety and depressive disorders is well known (Baldwin et al., 2002), and there is evidence for a serotonergic involvement in the pathogenesis of anxiety disorders. It has also been shown that various anxiety disorders can be treated successfully by antidepressants of the SSRI type (Westenberg, 1996; Kent et al., 1998; Schatzberg, 2000; Allgulander and Nilsson, 2001; Pollack et al., 2001; Figeo and Denys, 2009). In addition, tryptophan depletion leads to enhanced anxiety in carbon dioxide provoked panic in panic disorder patients (Schrüers et al., 2000). This effect was inhibited by administering L-5-hydroxytryptophan, which increases the availability of 5-HT (Schrüers et al., 2002). There is also evidence that IFN- α immunotherapy can increase anxiety in cancer patients, and this increase was reduced by antidepressant treatment with an SSRI (Musselman et al., 2001).

In animal models, only few studies analysed the impact of IL-2 on anxiety-like behaviour, and most of these studies yielded ambiguous results. Petitto et al. (2002) showed that IL-2/15R β knockout mice exhibited reduced anxiety-like behaviour in the EPM compared to heterozygous mice. On the other hand, there are studies in which neither acute nor repeated IL-2 treatments led to alterations in EPM behaviour (Petitto et al., 1997; Connor et al., 1998; Lacosta et al., 1999; Anisman et al., 2002). In our previous studies, we had shown that IL-2 directly injected into the striatum moderately (i.e. non-significantly) affected anxiety-like behaviour in the EPM (Pawlak and Schwarting, 2006), and significantly in an open field test (Karrenbauer et al., 2009). Our present experiment focused on effects of peripherally-administered IL-2 on anxiety-like behaviour. We assumed that IL-2, when tested 2 h after injection, in addition to its effects on depressive-related behaviour, should also affect anxiety-like behaviour. Contrary to our hypothesis, systemically injected IL-2 showed no effects on typical EPM measures of anxiety-like behaviours and activity parameters, indicating that the mechanisms which led to its effectiveness in the FST are at least distinct from those in the plus-maze.

4.4. Homeostatic measures for sickness behaviour

Dependent on the type of cytokine, these molecules can lead to symptoms of sickness behaviour, which can partly resemble depression, including fever, anorexia, sleep disturbance, lack of motivation, etc. (Dantzer et al., 2001). This behavioural pattern is also well known in animals at the onset of febrile infectious diseases, with behavioural characteristics like reduced grooming, lethargy, anhedonia, and anorexia (Hart, 1988). However, studies that analysed the ability of IL-2 to induce sickness behaviour are rare. Only Pauli et al. (1998) reported sickness after IL-2 treatment, but these findings are not comparable to ours (e.g., administration site, doses, not species-specific IL-2). However, Bhatt et al. (2005) showed no alterations in rectal temperature after a microinjection of IL-2 into the medial hypothalamus. Systemic IL-2 did also not affect body weight or food intake, using drug doses comparable to the present work (Migueluez et al., 2004). Supporting these latter findings, we measured body temperature before and after IL-2 treatment and found no changes compared to controls. The negative findings of IL-2 on sickness behaviour are in line with those of our previous studies (Pawlak et al., 2003, 2005; Pawlak and Schwarting, 2006; Karrenbauer et al., 2009; Schneider et al., 2010a, 2010b). Therefore, we assume that our behavioural findings in the FST are not due to sickness behaviour, but to more specific neuromodulatory alterations in motivation and emotion (Vitekovic et al., 2000; Frenois et al., 2007).

In summary, our experiments demonstrate that peripherally injected IL-2 can modulate neurochemical (5-HT, and partially DA) and behavioural parameters (depressive-related behaviour). These data are the first to establish that IL-2 decreases cortical 5-HT activity in a specific time profile, which may be informative for further studies. The fact that we observed IL-2 induced effects on depressive-related behaviour 2 h after injection, but not acutely, is suggested to be mediated, at least partially, by such serotonergic mechanisms.

Acknowledgements

This work was supported by grants from the Project Based Personnel Exchange Programme (0940042882 from the NSC and D/05/06869 from the German Academic Exchange Service DAAD), the German Research Foundation (DFG PA 818/4-1 and DFG Hu 306/27-2), and by funds of the University of Erlangen. The authors thank Anselm Crombach and Gabi Kütz for assisting in the collection of the behavioural data.

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